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Positive and negative chemotaxis and effect of drugs on *Balantidium coli* Stein

Chemotaksja dodatnia i ujemna oraz wpływ leków na *Balantidium coli* Stein

Free-living protozoans may show positive chemotaxis to compounds which (within the concentrations investigated) exert an injurious and even deadly effect on protozoa (Jennings 1899, Barratt 1905, Dogiel 1905, Dryl 1952, and others). Less known, or unexplored, are chemotactic reactions of parasitic protozoans, especially with respect to the compounds killing parasites. This problem may not only have a theoretical importance, but also a practical one, the more so, with reference to the locally applied protozoacidal drugs.

The aim of this work was to investigate the chemotactic reaction and to examine the susceptibility of *B. coli* to several protozoacidal compounds*. An orginal way of the quantitative determination of chemotaxis was worked out as no sufficiently reliable methods for the investigation of chemotaxis were available.

Methods

The methods for the examination of chemotaxis applied by Pfeffer, Barratt 1905, Jennings 1899, Dryl 1952, and others permit only the approximate, qualitative evaluation of this reaction. The results obtained by means of these methods may only give the approximate values and may not be analysed in point of the statistical significance. Neither the new method of Dryl 1959 (published when the present experiments were finished) is exactly satisfactory for solving these difficulties.

The procedure, aiming at the quantitative determination of the positive chemotaxis of balantidia, was already partly related at the Congress of Polish Anatomists and Zoologists (1959). A slide was covered with solid paraffine. Two round fields, 8 mm in diameter, were cut out in the layer of paraffine. Both fields were joined together along a segment 3 mm in length.

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0.05 ml of a suspension of the washed out protozoa in 0.85% natrium chloride solution and 667×10^{-5} M phosphate buffer, pH 6.8, was transferred on one of the fields. On the other field, 0.05 ml of the examined compound in 0.85% natrium chloride solution and 667×10^{-5} M phosphate buffer, pH 6.8, was placed. The slide was put into a damp chamber, at 37°C. After 10 minutes the connection between the two drops was interrupted by means of a cover-glass (or the like) placed vertically along the segment joining both of the fields. The protozoa were killed by addition of mercuric chloride solution (0.001%) into each drop. The number of protozoa on each of the fields was determined under the stereoscopic microscope (type M 110 PZO), magnification 85. The experiment was repeated with different concentrations of the compound examined preferably in the increasing geometrical progress.

The lowest concentration of the investigated compound causing positive chemotaxis (positive chemotactic value of Kagan), is not an adequate measure of chemotaxis. At the same threshold value, namely the course of the phenomenon may not be identical. This fact may be reflected in the different slope of dose-effect line. The more suitable way of estimation of the positive chemotactic action of the compound, might be the concentration (ED_{50}) of its solution, into which 50% of the investigated protozoa flows in within 10 minutes. As it was stated in these experiments, the dose-effect curve in the case of chemotaxis of protozoa consists of one ascending and one descending part, both being symmetrical. Hence, two concentrations, ED_{50} min. and ED_{50} max., on which a half of all protozoa reacts, might be possible. It is therefore proposed to accept the minimal solution concentration (ED_{50} min.) on which 50% of protozoa react as the comparable measure of the chemotactic action of the compound. It is calculated using regression equation for the relation between the percentage (N) of chemotactically positively reacting individuals and logarithms (c) of the corresponding concentrations of the examined solutions, according to the transformed Fisher's formula:

$$c_{50}\ \text{min.} = \bar{c} + k\ (50 - \bar{N})$$

where c_{50} min. = log ED_{50} min., $k = \dfrac{\sum (c - \bar{c})}{\sum_N (c - \bar{c})^2}$; $\bar{N}$ and $\bar{c}$ mean values.

In these calculations the ascending part only of the dose-effect curve is taken into account, i.e. the range in which the increase of the concentrations is followed by the increase of percentage N. When N = 0 for several concentrations, then only one, the highest, concentration value c_0 causing no chemotaxis, is used in the calculations.

It is proposed to accept the range $<ED_0$ min., ED_0 max.$>$ for the definition of the theoretical range of the positive chemotaxis. The lowest concentration (ED_0 min.), causing no positive chemotaxis of the studied compound, may also be obtained from the regression equation, to wit:

$$C_0\ \text{min.} = \bar{c} - k\bar{N}$$

Yet, the upper limit of concentrations causing the positive chemotaxis (ED_0 max.) is proposed to be calculated according to the equation:

$$c_0\ \text{max.} \approx 2\ cN\ \text{max.} - c_0\ \text{min.}$$

where c_N max. is a logarithm of a concentration causing the highest chemotaxis (N max.).

The negative chemotaxis was investigated in a quite similar manner. The suspension of protozoa in a solution of the examined compound, containing 0.85% natrium chloride solution and 667×10^{-5} M phosphate buffer pH 6.8, was placed on one of the fields of a slide, and 0.85% natrium chloride solution in the same buffer on another. After 10 minutes the connection between the drops was interrupted and the number of protozoa on each field was counted as before. The experiment was repeated with different concentrations of the examined compound. The lowest concentration (ED_{50} min.), causing the negative chemotaxis at 50% of protozoa, was also accepted as a measure for the negative chemotaxis. The range of chemotaxis is characterised by an interval $<ED_0$ min., ED_0 max.$>$.

The conditions under which the chemotaxis is being investigated according to the described method, may in certain limits be changed. The number of protozoa flowing into the investigated solution is influenced distinctly by the length of a segment joining both fields, cut out in the paraffine layer. In the experiments with the compound causing the positive chemotaxis, the percentage of the reacting balantidia, when the length of the segment joining both fields was 1, 2 and 3 mm., was respectively: $11.1 \pm 2.75\%$*, $40.4 \pm 4.59\%$, $57.4 \pm 4.48\%$. The influence of time within which the experiment is being conducted is also very important. Into the same solution, after 1, 5 and 10 minutes, have flown in: $6.6 \pm 2.3\%$, $27.9 \pm 4.05\%$ and $57.4 \pm 4.48\%$ of protozoa, respectively. In the control experiments with the buffered 0.85% natrium chloride solution, the other concentration, higher than ED_{50}, for instance ED_{75}, may also be accepted as a measure for chemotaxis. It depends on the chemotaxis value. Under conditions of this work, the percentage of balantidia flowing into the buffered 0.85% natrium chloride solution was from 0.0 ± 0.00 to $5.6 \pm 3.47\%$, the mean value being $2.97 \pm 0.46\%$.

As to the mutual intermixing of solutions on the contact area, it was stated using a dye, that after 10 minutes at 37° the dislocation of the limit of the coloured solution, containing no protozoa, was 1.7 ± 0.17 mm, whereas in the presence of balantidia it was 2.0 ± 0.29 mm.

The reproducibility of the results obtained by the described method is quite satisfactory. It was stated, when examining the positive chemotaxis of the *Balantidium coli* Stein strain No. 2 to the same compound within the interval of 9 months, that the differences between the results have not exceeded the half of the standard error at the chemotaxis value from 36 to 54%.

The susceptibility of balantidia to drugs was tested as follows: 0.02 ml of the suspension of washed out balantidia in 0.85% natrium chloride solution and 667×10^{-5} M phosphate buffer pH 6.8, have been added to 0.1 ml of a solution of the examined compound in the same solvent. The slide was placed in moist chamber at 37°. After 10 minutes, the number of plasmolysed individuals, magnified 100 times, was counted under the microscope on the

* Mean value $\pm$ the standard error.

suitably prepared slide, and, after killing the protozoa with a 0.001% mercuric chloride solution, their general number was determined. Basing on the results of the experiments carried out with different concentrations of the investigated compound, the concentration (LD_{50}), killing 50% of protozoa, was calculated from the regression equation (as indicated above) and the interval $<LD_0 - LD_{100}>$ as well. For several N equal 0 or 100%, only one highest c_0 or lowest c_{100} was taken into consideration in the calculations. The preliminary results of the experiments on the susceptibility of balantidia to poisons, conducted under somewhat modified conditions, have been presented on the VIth Congress of Polish Parasitological Society (1958). The strain *Balantidium coli* No. 2 applied in these experiments was cultivated from the swine large intestine contents in this Department by Rostkowska in 1956. It was kept on Pavlova's medium at 37°, pH 6.8, and transferred every 2 days. Protozoans used in the experiments were washed out twice with 0.85% natrium chloride solution containing phosphate buffer pH 6.8, profiting by the sedimentation of balantidia to the bottom of the test-tube. Protozoans applied in the experiments were derived from the two-days culture.

As well initial solutions, as the dilutions of: atebrin [3-chloro-7-methoxy--9-(1-methyl-4-diethylaminobuthylamino)-acridine], ethanol, chinosol (8-hydroxychinoline potassium sulphate), mercuric chloride, phenol, neosalvarsan (Neosalvarsan Bayer), potassium permanganate, penicillin (Penicillinum crystallisatum, potassium salt of penicillin G — Zakłady Farmaceutyczne in Tarchomin), streptomycin (Streptomycinum, Zakłady Farmaceutyczne in Tarchomin), and formaldehyde, were prepared in 0.85% natrium chloride solution and phosphate buffer pH 6.8. Aureomycin (Aureomycin Hydrochloride Lederle), sulphathiazole (sodium salt of 2-(p-aminobenzene-sulphonamido)--thiazole) and cupric sulphate have been dissolved in 0.85% natrium chloride solution only, for they were not soluble in buffer solution.

Results

The described investigations showed that amidst 13 protozoacidal drugs 10 couse the positive chemotaxis of *Balantidium coli* to wit: potassium permanganate (ED_{50} min. $= 137.9 \times 10^{-5}$ M), cupric sulphate (66.7×10^{-5} M), formaldehyde (2.8×10^{-5} M), phenol (116.9×10^{-5} M), ethanol (2814×10^{-3} M), atebrin (81.9×10^{-5} M), chinosol (144.5×10^{-5} M), neosalvarsan ($649.3 \times \times 10^{-5}$ M), aureomycin (1814×10^{-5} M), streptomycin (503.8×10^{-3} M).

Mercuric chloride, sulphathiazole, penicillin (Table III) caused no positive chemotaxis in the examined concentrations. The concentrations of the solutions on which the balantidia react with positive chemotaxis varied in wide ranges: from 0.9×10^{-5} M (formaldehyde) to 14.62 M (ethanol). Likewise, the range of concentrations causing positive chemotaxis was different for the particular compounds (Table I and II). The limit concentrations ratio $\frac{(ED_0 \max)}{(ED_0 \min)}$ which is characteristic for this range, varied between 4° (cupric sulphate) and 390 (atebrin).

For the majority of the examined compounds, the concentrations (ED_{50} min.),

on which 50% of protozoa react, was higher than the concentration (LD_{50}) killing half of the protozoans (Table I and II), except formaldehyde, atebrin and chinosol. This fact was stated when comparing the positive chemotaxis with the susceptibility of balantidia to the same compounds. The threshold concentration (ED_0 min. or LD_0) was usually lower for the positive chemotaxis than for the protozoacidal action of the compound examined. Of a practical significance may be the fact, that the concentrations of: potassium permanganate (74.6×10^{-5} M), cupric sulphate (49.6×10^{-5} M), phenol ($97.6 \times \times 10^{-5}$ M), ethanol (5918×10^{-3} M), chinosol (555.9×10^{-5} M), neosalvarsan (574.4×10^{-5} M), aureomycin (561.6×10^{-5} M) and streptomycin ($160.3 \times \times 10^{-3}$ M) lethal for 100% of protozoans (LD_{100}), caused still positive chemotaxis of balantidia. For formaldehyde only, the ranges of chemotactical (0.9×10^{-5} — 4.6×10^{-5} M) and toxic (40.8×10^{-5} — 260.0×10^{-5} M) action exclude.

Table I

Effect of drugs causing positive and negative chemotaxis

Kind of \ Drug			Potassium permanganate	Cupric sulphate	Formaldehyde	Phenol
Chemotaxis	positive	ED_{50} min.	137.9×10^{-5} M	66.7×10^{-5} M	2.8×10^{-5} M	116.9×10^{-5} M
		range (ED_0 min.-ED_0 max.)	37.4×10^{-5} — 267.1×10^{-5} M	20.2×10^{-5} — 79.5×10^{-5} M	0.9×10^{-5} — 4.6×10^{-5} M	17.1×10^{-5} — 585.3×10^{-5} M
	negative	ED_{50} min.	7440×10^{-5} M	23.5×10^{-5} M	1474×10^{-5} M	7.2×10^{-5} M
		range (ED_0 min.-ED_0 max.)	1.2×10^{-5} — 1425×10^{-5} M	3.4×10^{-5} — 186.3×10^{-5} M	32.6×10^{-5} — 592.3×10^{-5} M	2.5×10^{-5} — 17.8×10^{-5} M
Susceptibility		LD_{50}	52.6×10^{-5} M	27.3×10^{-5} M	103.0×10^{-5} M	44.1×10^{-5} M
		range (LD_0 - LD_{100})	37.0×10^{-5} — 74.6×10^{-5} M	15.0×10^{-5} — 49.6×10^{-5} M	40.8×10^{-5} — 260.0×10^{-5} M	19.9×10^{-5} — 97.6×10^{-5} M

Potassium permanganate (ED_{50} min. = 7440×10^{-5} M), cupric sulphate (23.5×10^{-5} M), formaldehyde (1474×10^{-5} M), phenol (7.2×10^{-5} M), sulphathiazole (26.96×10^{-3} M) and penicillin (0.56×10^{-3} M) caused negative chemotaxis in *Balantidium coli*. The concentrations of solutions on which balantidia reacted chemotactically negatively, varied in wide ranges from $1.2 \times \times 10^{-5}$ M (potassium permanganate) to 0.536 M (sulphathiazole). The threshold concentration (ED_0 min.) causing the negative chemotaxis was lower than the threshold concentration (ED_0) of protozoacidal action of all compounds causing the negative chemotaxis (Table I and II). In the case of potassium permanganate, cupric sulphate, formaldehyde and sulphathiazole the ranges of chemotactical and toxic effect have common regions. It must be emphasized,

Table II

Effect of drugs causing positive chemotaxis only

Kind of reaction / Drug			Neosalvarsan	Ethanol	Atebrin
Chemotaxis	positive	ED_{50} min.	649.3×10^{-5} M	2814×10^{-3} M	81.9×10^{-5} M
		range (ED_0 min. - ED_0 max.)	45.4×10^{-5} – 13262×10^{-5} M	615.6×10^{-3} – 14620×10^{-3} M	10.2×10^{-5} – 3900×10^{-5} M
	negative	value	0.0 ± 0.00 to 3.9 ± 2.25 % mean 1.8 ± 0.84 %	0.0 ± 0.00 to 9.5 ± 4.64 % mean 2.7 ± 1.77 %	1.9 ± 6.09 to 6.9 ± 3.34 % mean 3.8 ± 0.54 %
		examined concentration	83×10^{-5} – 833×10^{-5} M	417×10^{-3} – 8333×10^{-3} M	6.7×10^{-5} – 1667×10^{-5} M
Susceptibility		LD_{50}	208.3×10^{-5} M	2577×10^{-3} M	714.0×10^{-5} M
		range (LD_0 - LD_{100})	75.6×10^{-5} – 574.4×10^{-5} M	1123×10^{-3} – 5918×10^{-3} M	85.7×10^{-5} – 5939×10^{-5} M
Kind of reaction / Drug			Chinosol	Aureomycin	Streptomycin
Chemotaxis	positive	ED_{50} min.	144.5×10^{-5} M	1814×10^{-5} M	503.8×10^{-3} M
		range (ED_0 min. - ED_0 max.)	41.2×10^{-5} – 2974×10^{-5} M	297.5×10^{-5} – 3362×10^{-5} M	87.6×10^{-3} – 1028×10^{-3} M
	negative	value	0.0 ± 0.00 to 6.8 ± 4.74 % mean 3.4 ± 1.36 %	0.0 ± 0.00 to 4.9 ± 3.39 % mean 1.6 ± 0.69 %	0.0 ± 0.00 to 11.1 ± 4.26 % mean 3.9 ± 1.58 %
		examined concentration	58×10^{-5} – 583×10^{-5} M	83×10^{-5} – 833×10^{-5} M	33×10^{-3} – 417×10^{-3} M
Susceptibility		LD_{50}	210.4×10^{-5} M	221.3×10^{-5} M	157.5×10^{-3} M
		range (LD_0 - LD_{100})	79.6×10^{-5} – 555.9×10^{-5} M	87.2×10^{-5} – 561.6×10^{-5} M	126.1×10^{-3} – 160.3×10^{-3} M

that the determination of the upper limit (ED_0 max.) of negative chemotaxis by means of this method may offer certain difficulties owing to the simultaneous toxic action of a compound, especially when the chemotaxis value is small, as it is the case for formaldehyde and phenol. For the compounds causing positive and negative chemotaxis, the ranges of concentrations are separate only in the case of phenol (Table I). Among the compounds examined, it was only mercuric chloride which provoked neither negative, nor positive chemotaxis.

Conclusions

1. A new method for the quantitative determination of the positive and negative chemotaxis was worked out. It is proposed to accept the minimal solution concentration (ED_{50} min.) provoking a chemotaxis at 50% of protozoans as a comparable measure of the reaction.

Table III
Effect of drugs causing no positive chemotaxis

Kind of reaction / Drug			Mercuric chloride	Sulphathiazole	Penicillin
Chemotaxis	positive	value	0.0 ± 0.00 % mean 0.0 ± 0.00 %	0.0 ± 0.00 to 1.8 ± 1.89 % mean 0.3 ± 0.30 %	0.0 ± 0.00 to 10.6 ± 3.85 % mean 2.6 ± 1.66 %
		examined concentration	0.1×10^{-5} – 2×10^{-5} M	3.9×10^{-3} – 117.5×10^{-3} M	1×10^{-3} – 200×10^{-3} M
	negative	ED_{50} min.	no ED_{50} value 7.1 ± 3.95 to 9.7 ± 4.16 % mean 8.3 ± 0.91 % examined concentration 0.08×10^{-5} – 1.3×10^{-5} M	27×10^{-3} M	0.56×10^{-3} M
		range (ED_0 min. - ED_0 max)		7.95×10^{-3} – 535.7×10^{-3} M	0.55×10^{-3} – 1.27×10^{-3} M
Susceptibility		LD_{50}	0.732×10^{-5} M	66.74×10^{-3} M	134.4×10^{-3} M
		range (LD_0 - LD_{100})	0.373×10^{-5} – 1.44×10^{-5} M	37.6×10^{-3} – 118.4×10^{-3} M	121.4×10^{-3} – 148.9×10^{-3} M

2. Factors, and their concentrations which cause positive (see p. 7) or negative (see p. 8) chemotaxis of *B. coli* (see Table I, II, and III, too), were determined.

3. Protozoacidal and positive chemotaxis provoking ranges of concentrations have partly coincided at all compounds, except of phenol. Protozoacidal and negative chemotaxis causing ranges of concentrations have overlapped in the case of potassium permanganate, cupric sulphate, formaldehyde, and sulphathiazole, and excluded themselves only in the case of phenol and penicillin (see Table I, II, and III).

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STRESZCZENIE

1. Opracowano nową metodę ilościowego oznaczania chemotaksji dodatniej i ujemnej. Proponuje się przyjęcie minimalnej koncentracji roztworu (ED_{50} min.) wywołującej chemotaksję 50% pierwotniaków jako porównawczej miary reakcji.

2. Określono czynniki i ich koncentracje, które wywołują dodatnią (p. str. 110) lub ujemną (p. str. 111) chemotaksję *B. coli* (p. również tab. I, II i III).

3. Zakresy stężeń, wywołujące chemotaksję dodatnią i działające zabójczo na pierwotniaki, zachodziły na siebie dla wszystkich związków z wyjątkiem fenolu. Zakresy stężeń, wywołujące chemotaksję ujemną i działające zabójczo, zachodziły na siebie w przypadku nadmanganianu potasu, siarczanu miedzi, formaldehydu i sulfatiazolu, a wykluczały się dla fenolu i penicyliny.